26. The method according to claim 15, characterized in that said assaying of the enzymatic activity of said at least one reporter substance is done using fluorescence spectroscopy, especially confocal fluorescence spectroscopy.

27. The method according to claim 15, characterized in that said Gram-positive bacteria express lysostaphin immunity factor.

28. The method according to claim 15, characterized in that the fraction of reporter substances released by natural cell wall changes is determined.

29. The method according to claim 15, characterized int hat the fraction of reporter substances which are non-covalently bonded to the surface of Gram-positive bacteria is determined.

REMARKS

Claims 15-29 are presented hereby.

Claims 15 and 16 contain the subject matter of claims 1 and 2, respectively, claims 17 and 18 contain the subject matter of claim 3, and claims 19-29 contain the subject matter of claims 4-14, respectively, revised to more clearly define the invention.

The specification is amended, hereby, to correct identification of a trademark and add its generic description, as required in the Office Action. Marked up versions of the amended paragraphs are in the Appendix (attached hereto).

Reconsideration of the rejection under 35 USC 112, ¶2, is requested in view of the changes to the claims effected by the instant Amendment and in view of the following remarks.

With respect to the word "assaying," it is defined by the claims taken in view of the specification. Claims are to be given their broadest reasonable interpretation during prosecution, but the definition of a claim limitation given by the Examiner cannot be different than would be given by one of ordinary skill in the art. *In re Cartright*, 49 USPQ2d 1464 (Fed. Cir. 1999). Moreover, the Examiner's definition of a claim limitation cannot conflict with the definition given in the specification. *In re Zletz,* 13 USPQ2d 1320, 1322 (Fed. Cir. 1989). The examiner must use the specification definition in construing the claims for comparison with the prior art.

> When the applicant states the meaning that the claim terms are intended to have, the claims are examined with that meaning, in order to achieve a complete exploration of the applicant's invention and its relation to the prior art.

Zletz, 13 USPQ2d at 1322. Moreover, claim terms need not be "conventional" in the art, since a patent applicant is entitled to be his own lexicographer. *In re Castaing*, 166 USPQ 550 (CCPA 1970).

With respect to the "sequence LPXTG," there is no "sequence identifying number" to recite.

Claims 1-2, 5, 9-11 and 14 stand rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Samuelson. Reconsideration is requested.

Applicants submit that the statement of rejection suggests a possible misunderstanding of the invention as defined in the original claims (and in the present claims).

Samuelson teaches the cell surface display of heterologous proteins on *Staphylococcus carnosus* cells utilising a novel expression system. Samuelson discloses the following two expression vectors (see Fig. 1):

- pSPPmABPXM which encodes for a gene product consisting of a propeptide (PP), a 198-amino-acid-region, designated ABP (albumin binding protein), and cell wall anchoring regions X and M from staphylococcal protein A
- pSPPM3ABXM which encodes for a gene product into which a *P. falciparum*-derived peptide (M3) has been introduced between the propeptide and ABP

These recombinant surface-displayed proteins can be detected according to Samuelson by the following methods:

- Sandwich assay (see Fig. 3): Biotinylated HSA is allowed to bind to the reporter protein ABP. A streptavidin-alkaline phosphatase conjugate is added resulting in a non-covalent interaction between Biotin and Streptavidin. Excess conjugate is removed before the substrate solution ρ-nitrophenylphosphate is added and the color shift of the substrate is monitored.
- FACS analysis: The bacterial cells were probed with primary antibodies reactive with ABP and fluorescently stained with a fluorescein isothiocyanate-labeled secondary antibody. Thereafter, a FACS analysis was conducted. Please note that this embodiment does not make use of enzymes at all.

This clearly shows that Samuelson only discloses methods to display proteins on the surface of gram-positive bacteria and to detect them utilising binding assays (either binding of enzyme conjugates or antibodies).

The presently claimed invention, however, features a completely different approach: the identification of active substances like anti-infective drugs interfering with the mechanism of covalent bonding of polypeptides to the surface of gram-positive bacteria. According to the presently claimed invention, the skilled person is taught by the specification to identify such active substances as follows:

1. A sample of gram-positive bacteria is used. These bacteria produce an enzymatic reporter substance which can become covalently bonded to the surface of the bacteria. The ability of the enzymatic reporter substance to become surface-displayed can be achieved by utilising a hybrid between the enzymatic reporter and corresponding signalling and cell wall anchoring regions (e.g. a succession of N-terminal signal peptide, enzyme, LPXTG motif, hydrophobic and charged sequence segments). The surface-display of the reporter can be detected by virtue of its enzymatic activity as follows. Claim 1 teaches to use an enzymatic reporter which has different enzymatic activity being covalently bonded to the cell surface in comparison to being not covalently bonded. The change of enzymatic activity is in one embodiment due to a transition of the enzyme from an inactive form in its covalent surface-displayed state to an active form in its non-covalently bonded state. In case, an added substance does not interfere with the covalent bonding of polypeptides (e.g. enzymatic

reporter) to the cell-wall, the reporter is surface-displayed and inactive. In case, an added substance affects the covalent bonding mechanisms of polypeptides, the enzymatic reporter will not be covalently bonded to the surface of the bacteria. This state of the enzymatic reporter can be detected by its varied enzymatic activity.

2. It is according to claim 1 (and claim 15), also, possible to utilise gram-positive bacteria in which the enzymatic reporter is already covalently bonded to the surface. This scenario allows the detection of active substances which release polypeptides already covalently anchored in the cell wall, e.g., through the action of cell wall hydrolases. Again, the differences in the enzymatic activity of the reporter substance form the basis to identify active substances which result in a release of the enzymatic reporter from the surface of the bacteria.

Samuelson does not teach a method for identifying active substances which affect the covalent bonding of polypeptides to the surface of gram-positive bacteria. Neither does Samuelson disclose to covalently display an enzyme to the surface of the gram-positive bacteria as a reporter substance. The idea of utilising different enzymatic activities depending on the state of the enzyme (covalently bonded or not) to identify active substance is consequently completely missing.

Accordingly, Applicants submit that the rejection for alleged lack of novelty cannot be maintained against the rejected claims (or applied against the present claims).

Claims 3-4, 6, 12 and 13 stand rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Samuelson in view of Schneewind. Reconsideration is requested.

Schneewind discloses the existence of an at that time undescribed sorting mechanism that positions proteins on the surface of gram-positive bacteria. The signal involved in cell wall anchoring consists of an LPXTGX motif, a C-terminal hydrophobic domain, and a charged tail.

However, neither Samuelson nor Schneewind teach a method in which at least one reporter substance has a different enzymatic activity when not covalently bonded to the surface from that exhibited when it is covalently bonded to the surface of gram-positive bacteria. Neither do they teach the person skilled in the art to apply such a reporter substance in the search for active substances (like anti-infectives) affecting the covalent bonding of polypeptides to the surface of gram-positive bacteria.

Claims 7 and 8 stand rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Samuelson in view of Schneewind in further view of Strauss et al. Reconsideration is requested.

Strauss et al. teach the use of a proenzyme for in vivo immobilisation of enzymatically active polypeptides on the cell surface of *Staphylococcus carnosus*. As neither Samuelson nor Schneewind teach a method in which at least one reporter substance has a different enzymatic activity when not covalently bonded to the surface from that exhibited when it is covalently bonded to the surface of gram-positive bacteria, it would not have been obvious to use a proenzyme according to Strauss et al.

Accordingly, it would not have been obvious from Samuelson, Schneewind, and Strauss, taken either alone or in combination, that to apply an enzymatic reporter having the features of the invention as defined in the rejected claims (and the present claims), that interference with covalent

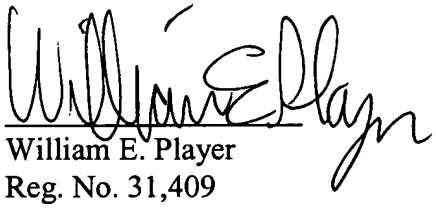
bonding results in a change in enzymatic activity of this reporter substance which can be used to identify active substances like anti-infective drugs.

Accordingly, Applicants submit that the rejections for alleged obviousness cannot be maintained against the rejected claims (or applied against the present claims).

Favorable action is requested.

Respectfully submitted,

JACOBSON·HOLMAN PLLC

By: 
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APPENDIX
VERSION WITH MARKINGS TO SHOW CHANGES

IN THE SPECIFICATION:

At page 19, replace the first full paragraph (beginning at line 3) with the following paragraph.

At different times of cultivation, the lipase activity released by the bacteria was determined in the culture superenatants. The cells were pelletized by centrifugation, the culture supernatants were taken off and, if necessary, stored on ice. Assays were performed in microtitration plates (100 µl) with glass bottoms. The Lipase assay buffer was constituted as follows: 10 mM $CaCL_2$, 0.05% [Triton] TRITON X-100 (alkylaryl polyether alcohol), 20 mM Tris/HCl, pH 8.0. As the fluorogenic dye substrate, 1,2-0-dilauryl-rac-glycero-3-glutaric acid resorufin ester (Sigma # D7414) was used. The substrate stock solution was prepared in 100% DMSO at 1 mg/ml and stored at -20 ^{0}C. Per measuring sample, 10 µl each of the culture supernatants was mixed with 80 µl lipase assay buffer and 10 µl substrate solution (10 µM final concentration). The conversion of the substrate was determined by fluorometry using a fluorescence (ELISA) reader, or by means of a fluorescence correlation spectrometer, such as ConfoCor™ (Carl Zeiss, Jena, and Evotec, Germany).

At page 21, replace the second full paragraph (beginning at line 5) with the following new paragraph:

The cell cultures (pTX15, pTX30, pTX15+pCXlif, pTX30+pCXlif) were first separated into cell pellets and medium by centrifugation. Then, the pellets were washed three times with BM and taken up in BM. Cell wall proteins released from pTX30 expressing cells by treatment with lysostaphin (80 µg/ml in BM; 30 min at 37°C) served as the reference. Dilutions from the samples were made. Thus, 95 µl of lipase assay buffer (10 mM $CaCl_2$, 0.1% [Triton] TRITON X-100 and 20 mM Tris-HCl, pH 8.5) containing the chromogenic lipase substrate p-nitrophenyl caprylate [Sigma] in a concentration of 5 mM was added to 5 µl each of the culture supernatants. The hydrolysis of the substrate was subsequently followed over 10 minutes at 30 °C photometrically using a microtitration plate (ELISA) reader (SpectraMax, Molecular Devices) or by means of fluorescence correlation spectroscopy with ConfoCor at a wavelength of 405 nm. The assays were performed in microtitration plates with or without a glass bottom.

At page 22, replace the fifth full paragraph (beginning at line12) with the following paragraph.

The cells were sedimented by centrifugation, the culture supernatants were taken off and, if necessary, stored on ice. Assays were performed in microtitration plates without a glass bottom. The lipase assay buffer (10 mM $CaCl_2$, 0.1% [Triton] TRITON X-100 and 20 mM Tris/HCl, pH 8.5) contained the chromogenic lipase substrate p-nitrophenyl caprylate [Sigma] in a concentration of 5 mM. Per measuring sample, 5 µl each of the culture supernatants was mixed with 95 µl lipase assay

buffer. The conversation of the substrate was determined by photometry using a microtitration plate (ELISA) reader.